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13. ABSTRACT ***(Maximum 200 Words)***

This study is the initial phase (the 1st year) of a feasibility study of a novel immunotherapeutic strategy for the treatment of breast cancer. The rationale is based upon recent findings that genes belonging to the pp32 family are differentially and alternatively expressed in most human breast cancers. In general, benign breast tissues express pp32, a tumor suppressor, whereas breast cancers express tumorigenic family members, including pp32r1 and pp32r2. Since pp32r1 and pp32r2 are expressed in nearly all breast cancers, but not in normal adult tissues, they may reasonably serve as targets for antigen-specific immunotherapy. The purpose of the study is to identify tumor-associated antigens (TAA) in pp32r1 and pp32r2, then test their suitability *in vitro* as immunotherapeutic targets in breast cancer. Currently, the second phase of (*in vivo*) feasibility is underway. If successful, the results may translate into eventual clinical trials of peptide vaccines or adoptive T cell therapy

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Introduction:

In the IDEA proposal, we proposed a feasibility study of a novel immunotherapeutic strategy for the treatment of breast cancer. The rationale is based upon recent findings that genes belonging to the pp32 family (Figure 1) are differentially and alternatively expressed in most human breast cancers. In general, benign breast tissues express pp32, a tumor suppressor, whereas breast cancers express tumorigenic family members, including pp32r1 and pp32r2. Since pp32r1 and pp32r2 are expressed in nearly all breast cancers, but not in normal adult tissues, they may reasonably serve as targets for antigen-specific immunotherapy.

Body:

Statement of Works:

Task 1. Identify, synthesize and test candidate peptides that could potentially bind to HLA class I molecules based on the coding sequence of pp32r1 and pp32r2. (Month 1-6)

Task 2. Screen *in vitro* for candidate pp32r1 & pp32r2 peptides that fulfill the requirements for TAA. (Month 7-12)

Task 3. Evaluate the pp32r1/pp32r2- specific cytotoxicity against a broad range of natural targets (established or primary breast cancer cell lines) to determine range of applicability.(Month 12-20)

Task 4. Evaluate *in vivo* immunogenicity of pp32r1 and/or pp32r2-derived TAAs in human breast cancer animal models. (Month 20-36)

In the first year of this project, we accomplished #1 and #2 Tasks defined by Statement of Work, but also worked to address an interesting suggestion offered by DOD BCRP Scientific Panel.

1) Task #1: Identify, synthesize and test candidate peptides that could potentially bind to HLA class I molecules based on the coding sequence of pp32r1 and pp32r2.

Using Bioinformatics and ImmunoGenetics tools, we analyzed the entire coding region of pp32, pp32r1 and pp32r2 genes for binding affinity with HLA-A*0201 molecule as well as the degradation pattern by proteasomal cleavages. The result of calculation shown (Table 1) that 19 motifs are potentially favorable of binding to HLA-A*0201 molecule with high affinity. To verify the prediction *in vitro*, HLA-A*0201+ TAP-deficient T2 hybridoma (ATCC) was pulsed with 50ug/ml of each peptide representing the motif (or control) and 5ug/ml of b2-microglobulin for 18hr at 37 C. HLA-A*0201 expression was then measured by flow cytometry using mAb BB7.2 (ATCC) followed by incubation with FITC-conjugated secondary antibody. Fluorescent index of HLA-A*0201 to each peptide can be determined as: (mean fluorescence with peptide - mean fluorescence without peptide) / (mean fluorescence without peptide). The result shown 10 out of 20 motifs is capable of binding to HLA-A*0201 in a concentration dependent manner (Table 1).

2) Task #2: Screen for candidate pp32r1 & pp32r2 peptides that fulfill the requirements for TAA.

In order to be qualified as a TAA, a motif has to be able to meet several criteria in addition to the binding to HLA-A*0201. These requirements include (i) the antigen can be naturally processed by tumor cells, (ii) it permits expansion of antigen-specific CTL; (iii) it is presented in a MHC-restricted fashion. CTL assay was carried out to test if the motifs identified in Aim#1 fulfill the requirements for TAA.

In brief, Cr^{51}-labeled target cells (T2 cells pulsed with peptide or cancer cell expressing pp32 family members) were incubated with various numbers of CTL effecter cells for 4 hr. Cr^{51}-release assays were performed in triplicate per condition using $5x10^3$ labeled target cells per well in a 96-well plate. Percent specific lysis will be calculated from CPM of (experimental result - spontaneous release)/(maximum release - spontaneous release). The results, summarized in Table 2, indicate that 2 out of 10 motifs fulfilled the above requirement as TAA.

3) Extra Task: Define pp32-related epitopes that is specific for CD4+ helper T Cell through MHC Class II.
During DOD BCRP Scientific Review for this Idea Proposal, the Reviewers expressed enthusiasium in whether pp32 family members are capable of activation of CD4+ helper T Cell through MHC Class II, in addition to the activation of CD8+ T Cell through MHC Class (addressed in task #1 and #2). To carry out this additional, we used several computer algorithms to select pp32r1 and pp32r2 sequences with potential promiscuous HLA-DR binding characteristics. We are currently testing the synthetic peptides corresponding to potential HLA-DR binding sequences for their capacity to stimulate $CD4^+$ T *in vitro* immunization.

Key Research Accomplishments:

We have identified two peptide motifs from pp32 family members, which fulfill the requirement to be TAAs. This study (mostly *in vitro*) provided bases for further evaluate the widely applicability of these TAAs in breast cancer immunotherapy (Aim #3) and *in vivo* validation in breast cancer animal models (Aim #4).

Reportable Outcomes:

The result of Specific Aim #1 and #2 were presented at 2002 Era of Hope Department of Defense Breast Cancer Research Program Meeting.
Yu, W., Jagun, A., Zhu, X., Jaffee, EM, & Bai, J. Identification of Candidate Tumor-Associated Antigens from pp32 Family Members. *Era of Hope* (BCRP): 3:54-2, 2002.

Conclusions:
We demonstrated *in vitro* that

(i) the oncogenic pp32 family members can be presented by HLA-A*0201,
(ii) the HLA-A*0201 cells bearing these motifs can be recognized and lyzed by pp32r1- or pp32r2- specific CTL in a MHC class I specific manner.

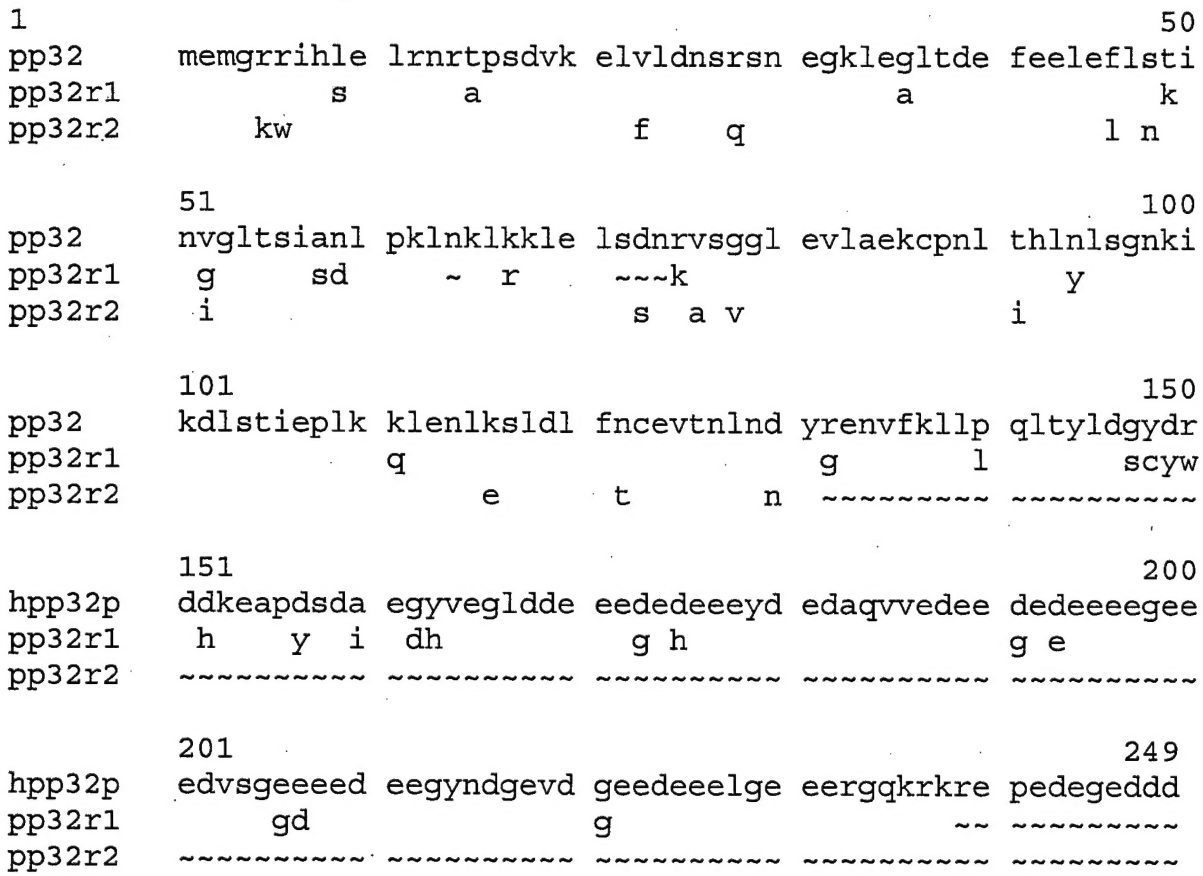

Figure 1. Alignment of pp32, pp32r1 & pp32r2 sequences.
Differences from the pp32 sequence are indicated underneath. The variant pp32r2 encodes a truncated protein (wavy lines indicate the truncated region).

Peptide	BIMAS	LpRep	FPEITHI	T2 Stabilization
0202-01	3499.535	3.37	26	+
0202-02	1591.602	2.46	22	-
0202-03	805.719	2.76	27	+
0202-04	681.542	3.54	18	+
0202-05	636.316	4.19	25	+
0202-06	481.542	6.90	27	-
0202-07	445.216	3.13	26	+
0202-08	432.319	4.87	21	-
0202-09	399.682	7.69	23	+
0202-10	379.216	5.81	13	-
0202-11	301.331	3.12	27	+
0202-12	281.542	3.47	22	-
0202-13	264.498	6.72	24	+
0202-14	226.014	3.54	20	-
0202-15	212.775	6.43	19	+
0202-16	172.752	6.81	21	+
0202-17	148.896	5.87	24	-
0202-18	139.730	6.72	19	-
0202-19	105.719	7.99	18	-
0202-20	103.362	6.79	21	-
MGA1	734.189	4.86	26	+

Table 1. Predicted HLA-A*0201 Binding Motifs and Their Ability to Bind T2 Cells.

Potential motifs was predicted by *BIMAS, LpRep, FPEITHI.*
The binding of Peptides to Human HLA-A2 was measured by T2 stabilization assay
Positive – calculated fluorescent index greater than 1.0.
Calculated fluorescent index = (Mean fluorescence with peptide - mean fluorescence without peptide)/(mean fluorescence without peptide)

Peptide	CTL Lysis*	Processing+	MHC I Restriction#
0202-01	+	n/a	n/a
0202-03	+++	Yes	Yes
0202-04	+	n/a	n/a
0202-05	+	n/a	n/a
0202-07	+++	Yes	Yes
0202-09	+	n/a	n/a
0202-11	-	n/a	n/a
0202-13	+	n/a	n/a
0202-15	-	n/a	n/a
0202-16	-	n/a	n/a
MGA1	+++	Yes	Yes
ID9	-	No	No

Table 2. Summary of CTL Assays for Motifs That are Capable of Binding to HLA-A*0201

Cytotoxicity Assay was carried out against Target cells:* T2 Cell +/- peptides

+ MCF-7 ($A2^+$,$pp32r1^+$,$pp32r2^+$)
LNCAP ($A2^+$,$pp32r1^-$,$pp32r2^-$)

MCF-7 (+/- anti-HLA-A2mAb)

Summary of Personnel Partially Supported by This Idea Award:

1) Jining Bai (PI)
Adetinuke Jagun (Technician)

CURRICULUM VITAE

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Education & Training:

1983-1988	B. Eng., Department of Engineering Physics, Tsinghua University, Beijing, P. R. China
1990-1996	Ph.D. / Graduate studies, Department of Biophysics, Johns Hopkins University Baltimore, MD
1996-1999	Post-doctoral Fellow, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD

Professional Experiences:

1985-1986	Instructor, Computer programming School of Professional Studies Tsinghua University, Beijing, P. R. China
1986-1988	Research assistant, Institute of Material Sci. & Tech., Tsinghua University, Beijing, P. R. China
1988-1990	Graduate studies, Department of Biol. Sci. & Tech., Tsinghua University, Beijing, P. R. China
1989-1990	Teaching assistant, Biology Lab, Department of Biol. Sci. Tsinghua University, Beijing, P. R. China
1991-1995	Pre-doctoral Fellow, Department of Embryology Carnegie Institution of Washington, Baltimore, MD
1992-1993	Teaching Assistant, Reproductive Physiology Johns Hopkins University.
1996-2000	Research Fellow, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD
2000-2001	Research Associate, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions Baltimore, MD
2001- 2002	Instructor, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions Baltimore, MD
2002-	Assistant Professor, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions Baltimore, MD

Bibliography:

Refereed Publications

Bai J, Brody JR, Kadkol SS, Pasternack GR. Tumor suppression and potentiation by manipulation of pp32 expression. *Oncogene*, 20 (17):2153-60, 2001.

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R., pp32 gene family. *Encycl. Mol. Medicine,* Vol.4, 2564-2565, 2001.

Kadkol SS, El Naga GA, Brody JR, Bai J, Gusev Y, Dooley WC, Pasternack GR. Expression of pp32 gene family members in breast cancer. *Breast Cancer Res Treat.* 68(1):65-73, 2001.

Kadkol SS, Brody JR, Pevsner J, Bai J, and Pasternack GR Modulation of oncogenic potential by alternative gene use in human prostate cancer. *Nature Medicine* 5(3):275-279, 1999.

Bai J., Pagano RE. Measuring the spontaneous transfer and transbilayer movement of BODIPY-labeled lipids in liposome vesicles, Biochemistry. (36):8840-8848, 1997

Ding, J. R., Zhou, X., Bai, J., Liu, B. X. Amorphous niobium monoxide films prepared by reactive evaporation, *J. Vac. Sci. Tech.* 8:3349-51, 1990.

Absracts

Yu, W., Jagun, A., Zhu, X., Jaffee, EM, & Bai, J. Identification of Candidate Tumor-Associated Antigens from pp32 Family Members. Era of Hope (BCRP): 3:54-2, 2002.

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R. pp32 Gene family at the crossroad of oncogenesis and tumor suppression. *Proc. of NCC,* 2:151, 2000.

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R. alterations in pp32 gene family – A novel molecular targets in breast cancer therapy. *Proc. of 4th NMC*, SGK Foundation, 4:22, 2000.

Kadkol, S. S., Saria, E. A., Brody, J. R., Bai, J. & Pasternack, G. R.. Analysis of alternative pp32 gene use in breast cancer. *J. of Mol. Diagnostics* 1:58, 1999

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R. Modulation of Oncogenic Potential *in vitro* and *in vivo* by pp32. *Proc. AACR.* 90:574, 1999

Kadkol, S. S., Brody, J. R., Bai, J. & Pasternack, G. R. Heterogeneous expression of members of the closely-related pp32 gene family in prostate cancer and adjacent normal prostate. *Am. J. Pathol.* 153:1660, 1998

Bai, J. , Kadkol, S. S., Brody, J. R., Chamberlin, M., Cheong, R. & Pasternack, G.R. Cell- Type Specific Suppression of Proliferation of Human Prostatic Adenocarcinoma Cell by a Novel Tumor Suppressor pp32. *Proc. AACR.* Supl. 10:A-10, 1998

Invention & Patents:

Pasternack, G.R. & Bai, J. Method of Treating Cancer by Restoration of pp32 Function, *United States Patent & Trademark Office (USPTO)*, #60/118667, 1999.

Grants & Contracts:

Current:

1) National Research Award — Komen Foundation — Principal Investigator
Active — (12/99-06/02) — $100,000 (annual direct)
Development of Novel Therapeutic Target and Approach for Breast Cancer
– Repairing Common Defects in Breast Cancer by Restoration of pp32.

2) IRG — JHMI — Principal Investigator
Active — (05/01-05/03) — $20,000 (annual direct)
Development of a Novel Transgenic Mouse Model for Human Prostate Cancer

3) Idea Award — DOD/CDMRP — Principal Investigator
Active — (10/01-10/04) — $100,000 (annual direct)
Identification of Widely applicable Tumor- Associated Antigens for Breast Cancer ImmunoTherapy.

4) Pilot Award — Breast Cancer SPORE/oncology — Principal Investigator
Active — (04/02-04/03) — $40,000 (annual direct)
HOXB7, Widely Applicable Targets for Immunotherapy against Breast Cancer.

Pending:

1) RO1 — NIH — Principal Investigator
Pending — (01/03-12/06) — $225,000 (annual direct)
Localization and Molecular Interaction of pp32 Family Members

Complete:

1) Concept Award — DOD/CDMRP — Principal Investigator
Active — (05/01-06/02) — $50,000 (annual direct)
Oncogenic Members of pp32 gene family, Widely Applicable Targets for Immunotherapy against Breast Cancer.

Honors & Awards:

Honored Student, Tsinghua University (1983-1988)
Outstanding College Graduate Award, National Education Commission of China (1988)
Winner of Natural Philosophy Competition, Tsinghua University (1990)
Travel Award, European Symposium in Signal Transduction (1991)
Carnegie Fellowship, Carnegie Institution of Washington (1990-1991)
Dean's Fellowship, Johns Hopkins University (1990-1995)
Pathology Fellowship, Johns Hopkins Medical Institution (1996-1999)
National Research Award, Susan G. Komen Breast Cancer Foundation (1999-2001)
Concept Award, Congressionally Directed Medical Research (2000-2001)
Idea Award, Congressionally Directed Medical Research (2001-2004)

Invited Lectures:

1) Alterations in pp32 Gene Family – A Novel Molecular Targets in Breast Cancer Therapy.
   The 4th National Mission Conference for Breast Cancer
   Washington D.C.
   September, 2000

2) pp32 Gene Family, Potential Therapeutic Targets for Breast Cancer and Prostate Cancer .
   National Cancer Institute
   Beijing, P.R. China
   October, 2000

3) pp32 Gene Family at the Crossroad of Oncogenesis and Tumor Suppression.
   The Cancer Congress 2000
   Beijing, P.R.China, October, 2000